Australia Antigen and Liver Function Tests Following Infectious Hepatitis

A Study of 111 Patients in Quest of Aids in Blood Donor Screening

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■ *An epidemic of infectious hepatitis involving 99 patients and employees of a state mental hospital revealed Australia antigen Au(1) to be absent from the blood of all but one of the subjects when tested at six weeks, three months, nine months and 12 to 18 months after onset of jaundice. The single patient with Au(1) at 12 months had no enzyme abnormality to indicate residual liver disease.*

If Au(1) is the virus of hepatitis these data would support the concept that persistent or long standing viremia is not a feature of epidemic hepatitis. Moreover, results of this study suggest that the Au(1) test should not be used to establish the absence of a past history of hepatitis in blood donors. These data do not establish the value of the Au(1) test in blood donors with active viremia, but do suggest that of 111 patients with recent hepatitis 1 percent had persistent antigenemia and 4 percent probably had circulating antigen antibody complexes and constituted a potential risk to recipients of their blood. The degree of risk to recipients from transfused blood of post-hepatitis patients without demonstrable Au(1) cannot be assessed.

ALTHOUGH IMPROVED TECHNIQUES in preparation of blood products and in selection of donors have reduced dangers to the recipient, the hazard of inoculation with hepatitis virus remains significant. This study was undertaken to evaluate one potential blood donor screening test in a selected population of which each member had recently acquired icteric infectious hepatitis.

The incidence of anicteric hepatitis has been estimated to be more than 100 times that of icteric hepatitis.[1] Hence exclusion of prospective donors with a history of jaundice offers about 1 percent effectiveness in donor screening. Mirick,[2] in a review of post-transfusion hepatitis and gamma globulin, stated that more practical than the ad-

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ministration of gamma globulin in reduction of risk of post-transfusion hepatitis would be elimination of all but essential transfusions and the use of great care in the selection of blood donors. Commercial blood sources are thought to be associated with a far greater incidence of hepatitis in the transfused patient than are volunteer sources[3,4,5] and it has been estimated that exclusion of the commercial donor could result in 90 percent fewer cases of transfusion hepatitis.[6] Critical shortages of blood preclude elimination of the commercial donor today although effective screening tests may make donor blood safer.

Thymol turbidity tests have been advocated for blood donor screening.[7,8,9] In 1966 Bolin, Chase and Alsever[10] described a test for detection of antibodies against viral hepatitis that was positive in 30 percent of donors. They indicated that such a test is impractical because the rejection of 30 percent of potential donors (only one-third may be carriers of virus) would make it almost impossible for blood banks to fulfill the demand for blood. It has recently been suggested that complement fixation tests for Australia antigen and specific anticomplementary activity can be used to screen large numbers of blood donors for hepatitis carriers.[11]

Australia antigen, Au(1), is a particle 20 microns in diameter with morphologic characteristics of a virus.[12] It is said to be intimately associated with a hepatitis virus and may be on the virus.[13] Recent evidence suggests that Au(1) antigen is an antigen of a hepatitis virus which can cause acute and chronic hepatitis or can persist in asymptomatic carriers.[12,14] Au(1) has been identified in some patients with chronic diseases in which there is an impairment of immune function; included are lymphatic leukemia,[15,16] leprosy,[17] Down's syndrome,[18,19,20] and patients with chronic renal disease treated with hemodialysis.[21,22] Au(1) is found in 5 to 20 percent of apparently normal populations of some tropical climates[23] but is present in less than 0.1 percent of the United States population.[24] Au(1) has been identified in the serum of patients with chronic active liver disease with cirrhosis.[25] While Au(1) has been found in the serum of some patients with acute viral hepatitis, its presence is usually transient, a few days or weeks.[13] In some instances of prolonged hepatitis it may persist for months or years.[26] It appears in the blood before signs or symptoms of acute hepatitis appear and remains in only about 7 percent of patients after recovery. The frequency of occurrence of Au(1) is greater in serum type hepatitis than in infectious hepatitis.[24] The antigen as determined by immunodiffusion testing has been identified in 41.1 percent of patients with post-transfusion hepatitis and 21.7 percent of patients with infectious hepatitis.[12,27] Other investigators report the incidence to be 34.1 and 13.1 percent respectively.[28] A recent study revealed Au(1) in 97 percent of 40 patients with serum hepatitis and absence of the antigen in 41 consecutive cases of infectious hepatitis.[32] In most instances Au(1) disappears as improvement occurs.[12,14] Hirshman et al[29] suggested that Au(1) appears to be a hepatitis virus and that a single virus group may be responsible for both infectious and serum hepatitis.

Identifying characteristics of two endemic forms of hepatitis were observed by Krugman and coworkers[30] in studies at Willowbrook State School in which newly entering inmates ingested material prepared from specimens obtained from diseased subjects. The short incubation agent, MS-1, caused apparent infectious hepatitis in 30 to 38 days while the long incubation agent, MS-2, caused a disease more like serum hepatitis in 41 to 108 days. Cross immunity between these agents was not demonstrated. Au(1) was identified in serum of only those patients inoculated with the MS-2 agent in a study by Giles et al.[31] Krugman and Giles[32] found that hepatitis-associated antigen was consistently present in serum from patients with MS-2 strain of serum hepatitis (SH) but was not present in MS-1 infectious hepatitis (IH). They also detected hepatitis-associated antigen earlier after a parenteral exposure to SH than after an oral exposure. The antigen appeared two weeks to two months before onset of jaundice and persisted for four months to 13 years in 35 percent of children. The SH antigen of Prince[33] is probably identical to Australia antigen.[34] In one study Au(1) was detected in 7.2 percent of blood donors. This rate is nearly that estimated for hepatitis carriers, 8.7 percent.[23]

Laboratory techniques for detection of Au(1), listed in order of increasing sensitivity or specificity, are agar gel diffusion, electronmicroscopic detection of sedimented antigen-antibody complexes, and complement fixation tests.[24] Fluorescent antibody techniques have revealed specific reactions between antibody to Australia antigen and an antigen on or within nuclei of liver cells.[35]

TABLE 1.—*Summary of Abnormal Results of Various Tests*

TEST	CHANGE	6 Wk. Test (111 pts.) ABNORMAL		3 Mo. Test (102 pts.) ABNORMAL		9 Mo. Test (92 pts.) ABNORMAL		12-18 Mo. Test (91 pts.) ABNORMAL	
		No.	Percent	No.	Percent	No.	Percent	No.	Percent
Total bilirubin	Increased	19	17	3	2.8	3	3.8	4	4.4
Ceph flocculation	Increased	73	66	60	59	46	51	38	42
Thymol turbidity	Increased	10	9	8	7.7	0	0	0	0
Total protein	Increased	10	9	18	17.1	5	5.4	5	5.5
Albumin	Increased	14	12.6	20	19.6	1	1.1	3	3.3
Albumin-globulin ratio (A/G)	Increased	30	27	28	28.5	7	7.6	9	10
A_1 globulin	Decreased	16	14.4	28	28.5	12	13	9	10
A_2 globulin	Decreased	26	23.6	15	14.7	12	13	6	6.6
B globulin	Decreased	32	28.7	28	28.5	14	15.2	13	14.2
Globulin	Decreased	3	2.7	3	2.9	1	1.1	1	1.1
S.G.O.T.	Increased	43	39	5	4.9	3	3.2	1	1.1
Alkaline Phos	Increased	46	41	47	46	35	38	44	48
Au(1) antigen by complement fixation		*3	2.7	0	0	0	0	1	1.1
Au(1) antibody by complement fixation		0	0	0	0	0	0	0	0
Au(1) antigen by immunodiffusion		0	0	0	0	0	0	0	0

*Anticomplementary

Methods

The study here reported was begun in 1960 by collecting blood from 111 patients with icteric hepatitis, 99 of whom were inmates or employees of a state mental hospital during an epidemic of infectious hepatitis in which 70 of the cases occurred in November and December of 1960. Initial specimens were obtained six weeks after onset of jaundice and repeated at intervals of three, nine and 12 to 18 months. Specimens were frozen and stored at −20° C until large groups could be analyzed together. One hundred two patients were available for follow-up at three months, 92 at nine months and 91 at 12 to 18 months. Attrition in the number of subjects was due to discharges and transfers.

Laboratory tests included serum glutamic oxalacetic transaminase, alkaline phosphatase, total bilirubin, cephalin-cholesterol flocculation, thymol turbidity, total protein and albumin and plasma protein paper electrophoresis. These tests were completed within six to twelve weeks after freezing of specimens. Tests for Au(1) included complement fixation for antigen, complement fixation for antibody and agar gel immunodiffusion for antigen. These were performed on serum specimens that had been maintained in the frozen state for nine to ten years. Standard complement fixation[11] and immunodiffusion[18] techniques were used. Positive and negative controls were used on each plate and each specimen was tested in duplicate.

Results

Test results are summarized in Table 1. Indices of liver function of groups three months, nine months and 12 to 18 months slightly exceeded normal ranges.

Complement fixation for Au(1) was positive in only one specimen (1:16)—and this was one year after clinical jaundice. There were no previous specimens on this particular patient. Three specimens were anticomplementary and all others were negative at the six-week period. Complement fixation tests for antibody to Au(1) were negative in all 502 specimens. Immunodiffusion tests for Au(1) were negative in all samples.

Discussion

All patients had clinical and biochemical evidence of infectious hepatitis at the onset of illness. All were jaundiced. All were considered by their physicians to have completely recovered before the third month serum specimens were obtained. There was no recurrence of jaundice in any patient during the period of study.

Interpretation of some results was made difficult because of exposure of most patients to tranquilizer drugs during observation. Persistence of

TABLE 2.—*Test, Methods and Normal Ranges Used in Study*

Test	*Methods*	*Normal Values*
Total bilirubin	Malloy-Evelyn	up to 1.6 mgm/100 ml
Cephalin-cholesterol flocculation	Hanger	0 to 2+ in 48 hours
Thymol turbidity	Shank-Hoagland	0 to 6 units
Alkaline phosphatase	King-Armstrong	0 to 11 units
SGOT	Reitman-Frankle	up to 40 units—males
		up to 35 units—females
Total protein	Kingsley	6.2 to 8.5 gms/100 ml
Albumin		3.5 to 5.5 gms/100 ml
Globulins		
$Alpha_1$	Paper electrophoresis*	0.2-0.4 gms/100 ml
$Alpha_2$		0.5-0.9 gms/100 ml
Beta		0.6-1.1 gms/100 ml
Gamma		0.7-1.7 gms/100 ml
Australia antigen by complement fixation	Shulman & Barker	Negative
Australia antibody by complement fixation	Shulman & Barker	Negative
Australia antigen by immunodiffusion	Allison & Blumberg	Negative

*The spinco analytrol and Durum cells were obtained from Beckman Instrument Corporation—Fullerton, California

a relatively high incidence of abnormal cephalin-cholesterol flocculation tests in each period (66 percent at six weeks, 59 percent at three months, 51 percent at nine months and 42 percent at 12 to 18 months) may have been influenced by tranquilizer drugs which 68 percent of the patients with elevated values had received. Seventy-seven percent of patients with elevated alkaline phosphatase levels received tranquilizers. Tranquilizer drugs included phenothiazine derivatives (prochlorperazine, thioridazine, fluphenazine, promazine, trifluoperazine, chlorpromazine and perphenazine) and non-phenothiazine derivatives (chlordiazepoxide and rauwolfia derivatives). Three or more drugs were prescribed on an alternating schedule. Only one form of drug was given at any one time.

Craddock[36] said that jaundice appears in about 1 percent of patients treated with chlorpromazine in mental hospitals and liver function tests results are similar to those found with obstructive jaundice (elevated alkaline phosphatase and transaminase levels). This jaundice may appear several weeks after discontinuance of the drug and the alkaline phosphatase may remain elevated after the serum bilirubin has returned to normal. Wailzkin[37] found no correlation between levels of serum bilirubin and alkaline phosphatase in chlorpromazine induced jaundice.

Reduction of values for globulin fraction was seen to be nearly equally divided between patients treated with tranquilizers and those untreated. Depression of all fractions of globulin in patients receiving long-term tranquilizer medication has been reported.[38]

Cephalin flocculation and thymol turbidity tests have almost always been normal in patients with jaundice due to chlorpromazine.[39] The low incidence of abnormal thymol turbidity levels (9 percent at six weeks and 8 percent at three months) does not support the findings of others.[7,8,9]

All three instances of anticomplementary tests for Au(1) were in six-week specimens, and subsequent specimens for each patient were nonreactive. Anticomplementary activity may be due to a combination of antigen and antibody[11] or may reflect the presence of nonspecific reactants. There was only one patient with a positive complement fixation test, and the enzymes were normal in this case. Au(1) was not detected by less sensitive immunodiffusion tests in any of those found to be anticomplementary. Au(1) antibody by complement fixation was not detected in a single case. These findings suggest that, if Au(1) appeared early in the acute phase of infectious hepatitis in these patients, early disappearance is rapid as recovery occurs.

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IMMEDIATE REPAIR FOR SECTIONED FACIAL NERVE

The patient has had a laceration of the face, and in exploring the wound you discover a sectioned facial nerve. What do you do?

"Immediate surgical repair is essential. The ends of the nerve can be approximated, especially if it's a clean incisional wound. It is much more difficult to try to repair a sectioned facial nerve after the area has been healed. Working through scar tissue makes it exceedingly difficult so I think that immediate repair at the time the laceration is sutured is indicated. The jagged lacerations are much more difficult to take care of than the clean incisional wounds, such as those from a knife or from glass, but they can be managed."

—FRANK D. LATHROP, M.D., Boston

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